

Original Research Article

Evaluation of Gingival Crevicular Fluid and Serum Levels of Interleukin-20 in Periodontal Health and Disease

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ABSTRACT

Periodontitis is a chronic inflammatory disease that causes destruction of tooth-supporting tissues. Interleukin-20 (IL-20) is a proinflammatory cytokine of the IL-10 family that is involved in some diseases; rheumatoid arthritis, psoriasis, and atherosclerosis. The purpose of this study was to evaluate the levels of IL-20 in gingival crevicular fluid (GCF) and serum of both chronic periodontitis patients and periodontal healthy subjects. Thirty systemically male subjects were categorized into group 1 (healthy) and group 2 (chronic periodontitis patients). The patients were clinically evaluated by; plaque index (PI), bleeding index (BI), probing pocket depth (PD) and clinical attachment loss (CAL). GCF and serum samples were obtained from all individuals to measure IL-20 levels by using of enzyme-linked immunosorbent assay (ELISA) technique. The levels of IL-20 in GCF were significantly higher in chronic periodontitis patients compared to healthy subjects ($P \leq 0.05$). In addition, there was a positive correlation between GCF-IL-20 levels and PD ($r = 0.800$, $P = 0.010$). While, the serum levels of IL-20 in chronic periodontitis patients were not significant in comparison to healthy subjects ($P \geq 0.05$). For Conclusion, significant increased levels of GCF-IL-20 in chronic periodontitis patients showed that IL-20 may have a role in pathogenesis of chronic periodontitis. Moreover, additional studies are required to clarify its role in periodontal diseases.

Keywords: IL-20, GCF, Serum, Chronic periodontitis

INTRODUCTION

Periodontal diseases comprise a group of inflammatory conditions of the periodontium that are initiated by specific species of microorganisms and mainly categorized into gingivitis and periodontitis are among the most common human infectious diseases. ^[1] Periodontitis occurs due to the challenge between periodontal pathogens and the host immune defenses. ^[2] Cytokines play a critical role in mediating the inflammatory processes and tissue homeostasis underlying periodontitis. Widespread research has been conducted to

exhibit the expression and changes of various kinds of cytokines in normal periodontal tissues and pathological conditions. ^[3]

Several proinflammatory cytokines are involved in pathogenesis of periodontitis such as IL-1 β , IL-6, TNF- α and IL-17, while, there are anti-inflammatory cytokines as transforming growth factor- β (TGF- β) and IL-10 down regulate the inflammatory process. ^[3,4]

The IL-20 is pleiotropic inflammatory cytokine; a member of IL-10 family is expressed in monocytes, epithelial

and endothelial cells, IL-20 can be induced by lipopolysaccharides, hypoxia or oxidized low density lipoprotein, IL-20 not only acts as a chemoattractant and angiogenesis factor but also induces other chemokines and angiogenesis factors. [5,6]

Bone resorption is considered a major pathological issue in chronic inflammatory diseases such as rheumatoid arthritis, osteoporosis and periodontitis. [5] In 2011, Hsu et al. reported that the anti-IL-20 monoclonal antibody inhibits the differentiation of osteoclasts and is a potential therapeutic for protecting against osteoporotic bone loss. [7] Recently, Zhang et al 2017 recorded that the topical application of recombinant IL-20 restored corneal wound healing to baseline levels while neutrophil recruitment remained low in murine model. They concluded that IL-20 plays a beneficial and direct role in corneal wound healing while negatively regulating neutrophil and platelet infiltration. [8]

According to available literature, there are no studies on relation of IL-20 and periodontitis except one study that showed that the serum levels of IL-20, IL-6 and TNF- α were significantly higher in patients with moderate and severe periodontitis or patients with chronic obstructive pulmonary disease (COPD) than those of their respective controls. [9] The present study aimed to evaluate gingival crevicular fluid and serum levels of IL-20 in healthy and chronic periodontitis individuals.

MATERIALS AND METHODS

Subjects

Thirty male individuals aged between 24 and 56 years were selected from Umm Al-Qura Dental Teaching Hospital after ethical approval and informed consent was obtained. They were categorized into two equal groups: group 1 (15 healthy subjects, with mean age 28.6 ± 4.85) and group 2 (mild to moderate chronic periodontitis patients, 35.13 ± 7.14). Subjects who participated in this study were generally systemically free and did not receive any periodontal therapy during the

past 6 months, Patients were not on any medication such as antibiotics and inflammatory drugs for last 3 months, and smokers were excluded from the study. Prior to GCF & serum samples collection, the following periodontal parameters were taken: Plaque index (PI), [10] Gingival index (GI), [11] Pocket depth (PD), and clinical attachment loss (CAL). [12]

GCF sampling

Before GCF collection, the supragingival deposits were removed gently without any trauma to the gingival crevice. Two μ l of GCF were collected using micropipette capillary tubes from the right maxillary quadrant to avoid salivary contamination. Then, GCF was diluted with 200 μ l of phosphate buffered solution in 1.5 ml Eppendorf tube and stored under at -80 °C until laboratory investigation.

Serum samples

Blood was collected in 3 ml tubes from all subjects after obtaining their consent. Blood samples were left undisturbed at room temperature for 30 min then centrifuged for 3000 rpm for 5 min to separate the serum. The serum was stored at -80 °C until laboratory analysis.

ELISA technique

Human IL-20 enzyme-linked immunosorbent assays (ELISA) kit was purchased from Abcam, UK. IL-20 was measured by ELISA according to manufacturer instructions.

Statistical analysis

Statistical analysis was done by using SPSS (statistical package for social science) program version 22. The quantitative data were presented in the following mean and standard deviation. Unpaired t test was used to test quantitative data; in addition, Pearson correlation was used to study correlation between periodontal indices versus to GCF-IL-20 levels. Significance was considered when P value ≤ 0.05 . The statistical data was tabulated and graphed by using Microsoft word 2015.

RESULTS

Evaluation of IL-20 levels (pg/ml)

The mean values of IL-20 levels in GCF in healthy subjects (G1) and periodontitis patients (G2) were 0.21 ± 0.66 and 1.25 ± 2.79 respectively (Table 1, Fig 1). The statistical analysis showed significant differences ($P \leq 0.05$). The mean values of serum levels of IL-20 in both groups were (0.41 ± 1.02 and 0.58 ± 1.66) correspondingly. The statistical comparison between the mean values of both groups was non-significant difference ($P \geq 0.5$). However, there was a positive correlation between pocket depth and GCF - IL-20 level in Group 2, ($r = 0.800$), ($P \leq 0.05$) (Table 2, Fig 1).

Table-1: Revealed the statistical differences of IL-20 levels between healthy and chronic periodontitis subjects.

	Groups	Mean \pm SD	T value	P value
GCF IL-20 levels	Group 1	0.21 ± 0.66	-1.146	0.015*
	Group 2	1.25 ± 2.79		
Serum IL-20 levels	Group1	0.41 ± 1.02	-1.00	0.363
	Group 2	0.58 ± 1.66		

Group 1= healthy individuals Group 2= Chronic periodontitis patients * = Significant

Table-2: Showed the statistical correlation between the mean values of GCF IL-20 and clinical parameters in group 2 (chronic periodontitis patients).

	GCF - IL-20 Levels (1.25 ± 2.79)	
	Pearson Correlation	Significance
Plaque index % (61.1 ± 0.06)	- 0.304	0.427
Bleeding index % (63.71 ± 0.23)	- 0.299	0.435
Pocket depth (2.2 ± 0.64)	0.800	0.010**
Clinical attachment loss (1.8 ± 0.52)	- 0.076	0.846

** = Highly significant

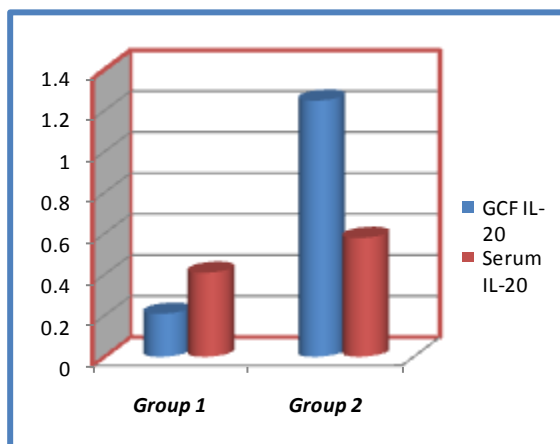


Figure-1: Revealed the mean values of IL-20 (pg/ml) levels in healthy and diseased groups.

Group 1= healthy individuals Group 2= Chronic periodontitis patients

DISCUSSION

Destruction of periodontal tissue is initiated and progressed through production of numerous virulence factors that may directly operate on tissue or indirectly by stimulating the immune and inflammatory reactions. Many bacteria in subgingival flora seem capable of damaging crevicular epithelium by releasing of toxic substances, which produced by *Porphyromonas gingivalis*, *Prevotella intermedia*, *A. actinomycetemcomitans*, *terponema denticula* and *Capnocytophaga species*. This would increase the permeability of the crevicular epithelium to bacterial products and possibly to the bacteria themselves. [13]

Periodontal pathogens produce metabolic by-products such as H₂S, NH₃, and fatty acids that are toxic to surrounding cells. Furthermore, bacterial constituents such as lipopolysaccharide (LPS) are capable of inducing bone resorption in vitro. [2-4] Constituents of the bacterial biofilm also stimulate host cells to produce proinflammatory cytokines, matrix metalloproteinases (MMPs) and arachidonic acid metabolites. These host products play catabolic activities, which can induce connective tissue and alveolar bone destruction. [14]

Interleukin-20 is a pleiotropic cytokine with potent inflammatory, angiogenic, and chemoattractive effects, all of which are characteristics of psoriasis, rheumatoid arthritis, and atherosclerosis. The previous involved diseases are characterized by the chronic inflammatory status similar to chronic periodontitis that also characterized by inflammatory angiogenic effect. [15]

Several studies documented strong relationship between periodontitis and rheumatoid arthritis. [16] Our hypothesis in this research was to study the levels of IL-20 in GCF and serum in healthy compared to chronic periodontitis patients. In the present study, the collection of GCS samples were collected by using micropipette capillary tubes and this

technique is adapted with some studies. [17,18]

In current study, the statistical analysis demonstrated that a significant increased differences of mean values of GCF- IL-20 in diseased group versus healthy individuals. This result shows that IL-20 may play a role in progression of periodontitis. This conception is supported by studies which reported local increased levels of IL-20 in synovial tissues and fluid. [19,20]

Probing pocket depth is considered an indicator for progression of periodontal inflammation rather than the severity of periodontitis. [21,22] Our results showed that significant correlation between GCF-IL-20 and probing pocket depth. This result may observe the role of IL-20 in progression of periodontal disease.

Here we found no significant difference in the levels of serum IL-20 in periodontitis patients compared to healthy individuals. This finding is inconsistent with study of Zuomin et al 2010, [9] they reported that serum levels of IL-20 were significantly increased in patients with moderate and severe periodontitis or patients with COPD than those of their respective controls. For clarification, the selected individuals in our study had mild to moderate status of periodontitis, and they were systemically medical free.

It is worthy to note that in this study the number of participants was low and the patients had mild to moderate chronic periodontitis. To overcome these drawbacks, we should include large count of patients with moderate to severe periodontitis in future study.

CONCLUSION

In conclusion, the current findings suggested that the IL-20 might have a role in the periodontal destruction of chronic periodontitis. Further investigations are required to clarify the specific contribution of IL-20 in production and progression of periodontal diseases.

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